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Procedia Food Science 1 (2011) 455 – 462

Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Study of adsorption isotherm and microbiological quality of fish meal type “piracuí"of Acari-Bodo (*Liposarcus pardalis*, Castelnau, 1855)

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Abstract

The Acari-Bodo (*Liposarcus pardalis*, CASTELNAU, 1855), is a freshwater fish of the family of acaris (Loricaridae). This species of fish is sold alive in shipping due to the rapid process of degradation after death which creates a repulsive odor, and render it impossible for consumption. However, it offers huge potential for technological development because it presents excellent acceptance in the market place. In addition to being consumed fresh, the muscle is also used for the manufacture of fish-meal, "piracuí", a product widely consumed in region, especially in inland areas. The Acari-Bodo, raw material of "piracuí", proves ideal for the manufacture of this product, because of its low fat content, and an excellent source of protein. Despite the nutritional advantages, this product shows problems during its production, in a traditional manner and rudimentary, with limited technological means, which imposes several restrictions from the point of view of health and trade. The critical points are related to poor hygiene in the handling of raw material and final product, materials and equipment used, packing, storage and marketing, besides the quality of the fish used. The aim of this study was to construct the adsorption isotherm for fish meal type piracuí "and cross the results with microbiological determination to fix the stability and storage conditions of the product. The "piracuí" showed type III isotherms and exponential behavior above 0.6 Aw. The product will have microbiological stability at Aw < 0.6 if humidity content will be below 10g/100g-1 d.b. The model with best fit (P and R^2) was BET. The product showed an absence of Salmonella, coagulase positive Staphylococcus, coliforms at 45°C, yeasts and molds. However, Aw below 0.6 occurred shortly halophilic bacteria growth. The results of this research provide data for the study of materials that can be used as packaging for storage of fishmeal type piracuí due this product to be frequently traded in the Amazon region.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: meal; isotherm; microbiological quality; fish

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2211 601X

doi:10.1016/j.profoo.2011.09.070

1. Introduction

The Acari-Bodo (*Liposarcus pardalis*), a freshwater fish of the catfishes and acaris family (Loricaridae) is consumed fresh, has a low lipid content and is an excellent source of protein. Its muscle is also used to manufacture a fishmeal called "piracuí" a product widely consumed in the Amazonian region [1].

The technique to prepare the "piracuí" is to grind the previously dried fish to reduce it into a powder and dry this material in oven called "nhaenpuna" or "yapuna", obtaining a product with flocculent texture. This product is mainly consumed by native people of Amazon region and represents an important source of protein, about 70% protein with optimum digestibility in the diet of the population, particularly by the low-income people [2].

Even with all these nutritional advantages, this product demonstrates some problems during its production, since it has remained virtually unchanged, that is, continues being held in a traditional manner and rudimentary, with limited technological , which imposes serious restrictions on point of view of health and trade. The critical points are related to poor hygiene in the handling of raw material and final product, materials and equipment used, potting, storage and marketing, besides the quality of fish used [2, 3].

The aim of this work was to prepare the fishmeal type "piracuí" from Acari-Bodó and build the adsorption isotherm of the manufactured product, linking it with microbiological results and therefore estimating the stability and storage conditions of this product.

2. Materials and Methods

2.1 Materials

We purchased 53 samples of Acari-Bodo (*Liposarcus pardalis*) in the city of Santarem, Pará, in the harvest period of the species, which covers the months of September and October. After gutting, 27.85 kg of fish was weighed in a balance (Filizola). Samples were stowed in a styrofoam box with ice in a proportion of 1:1 (fish: ice), transported by air to the city of Belém and sent to the Laboratory of Meat and Fish of the Food Engineering Faculty, UFPA. In the laboratory, the fishes were washed with chlorinated water (5ppm) to remove traces of gills, viscera and other parts of blood, packed in polyethylene bags, labeled and frozen at -18°C.

2.2 Preparation of fishmeal type "piracuí"

To obtain the "piracuí" fish meal, the fishes were placed in stainless steel containers into a water bath at the temperature of 95°C for 40 minutes. The temperature control was accomplished using a thermocouple and the cooking was conducted until loosen up the muscle of the carapace. Then the fishes were wrapped in polypropylene boxes at room temperature until cool down. The muscle was separated from the carapace and bones are manually pressed to remove the excess of water. In the pressed muscle NaCl (2% of the total weight) was added and it was ground into a meat processor (Wallita) for an uniform sample. For drying, the sample was divided into two portions, one of them was dried at 80°C and the other at 100°C, according to preliminary tests and Castro [2], in an oven with forced air (Quimis model Q 314 M122) up to moisture of 10% , as recommended by Brazil [4].

The drying time of the individual portions of 100g at temperatures of 80 and 100°C was five and seven hours respectively with a mixing every thirty minutes. Moisture control was done in an infrared balance (GEHAKA BG 200). After drying, the samples of "piracuí" were cooled down at room

temperature and packed in vacuum plastic bags of nylon/polyethylene, with low permeability to gases and water vapor, wrapped with aluminum foil and stored at room temperature for further analysis.

2.3 Microbiological analysis

Samples of muscle and fishmeal were analyzed for parameters required by the RDC No. 12 of January 2, 2001 [5], which are: Coliforms at 45°C, Staphylococcus coagulase positive, *Salmonella* sp and followed the methodology described by Vanderzant; Splittstoesser [6].

2.4 Adsorption isotherms

To obtain the adsorption data the desiccator method described by Assunção & Pena [7] was used. In semi-analytical balance, 1.5g of fishmeal type "piracuí" was weighed into capsules suited for the water activity measurement. Then, the capsules + sample were subjected to dehydration at room temperature (≈ 25°C) in desiccators with silica gel at the base, for a period of 24 hours, in order to achieve the lowest possible water activity. After this period, samples were transferred to a desiccator containing water at the base, at room temperature (≈ 25°C ± 1°C) to initiate adsorption.

In adsorption experiments, samples were withdrawn in duplicate at increasing times (pseudo-equilibrium), to determine the moisture content (mass difference), with the aid of a semi-analytical balance and Aw measurements were performed in a hygrometer (Aqualab 3TE). During all tests the samples were subjected to visual inspection, to monitor visibly noticeable changes as: caking (compaction), browning and fungal growth. The adsorption isotherm was constructed for 25°C, from the relationship between the moisture of the product and the corresponding Aw, was achieved using the program Statistica 5.0.

2.5 Prediction of adsorption isotherms

We tested eight mathematical models, three bi-parametric (Table 1) and five tri-parametric tests (Table 2), to predict the adsorption data of fishmeal type piracuí. The model fitting was performed using the software Statistica version 5.0 using the methodology of estimation of Quasi-Newton. The parameters used to evaluate the adjustments were: coefficient of determination (R^2) and the relative mean deviation (P), according to Equation 1.

$$P = \frac{100}{n}\sum\left|\frac{m\exp - mpre}{m\exp}\right| \qquad (1)$$

Table 1. Bi-parametric models used to predict adsorption isotherms.

Equation name	Model
Halsey (a)	$m = \left[\frac{-a}{\ln a_w}\right]^{\frac{1}{b}}$
Bet linearized (b)	$\frac{a_w}{(1-a_w).m} = \frac{1}{m_0.C} + \frac{(C-1)}{m_0.C}.a_w$
Oswin (a)	$m = a\left[\frac{a_w}{1-a_w}\right]^b$

m = moisture; mo = monolayer; A_w = water activity; a, b e C = constants
.(a) Iglesias and Chirife [8]; (b) Brunauer et al [9]

Table 2. Tri-parametric models used to predict adsorption isotherms.

Equation name	Model
GAB (a)	$m = \frac{m_0.c.k.a_w}{[(1-k.a_w).(1+(C-1).k.a_w)]}$
BET (a)	$m = \frac{m_0.c.a_w}{1-a_w}\left(\frac{1-(n+1).a_w^n + n.a_w^{n+1}}{1-(1-c).a_w - c.a_w^{n+1})}\right)$
Anderson (b)	$m = \frac{m_0.c.k.a_w}{[1+(c-2).k.a_w + (1-c).k^2.a_w^2)]}$
Anderson & Hall (b)	$m = \frac{m_0.c.k.a_w}{[1+(c-2.k).a_w + (k^2-c.k).a_w^2)]}$
Gascoyne & Pethig (b)	$m = \frac{m_0.c.k.a_w}{[1+(c-2.k).a_w + (k-c).k^2.a_w^2)]}$

m = moisture; mo = monolayer; A_w = water activity; a, b, c, k, n = constants.
(a) Park & Nogueira [10]; (b) Boquet et al [11]

3. Results and Discussion

3.1 Microbiological analysis

Table 3. Count of microorganisms in vacuum packed fishmeal type "piracuí" stored at room temperature.

Days	Coliform at 45°C (NMP/g)	Staphylococcus coagulase positive (UFC/g)	*Salmonella*	Molds and Yeasts (UFC/g)	Halophilic
0	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$1.5x10^3$
15	$< 1.0x10^1$	$< 1.0x10^1$	absence	$3.0x10^2$	$6.1x10^2$
30	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$4.5x10^2$
45	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$1.1x10^3$
60	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$6.0x10^2$
75	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$2.6x10^3$
90	$< 1.0x10^1$	$< 1.0x10^1$	absence	absence	$8.6x10^2$

Currently there are no microbiological standards established for fishmeal type "piracuí" in Brazilian law, so we used the standard microbiological referring to fish dry and / or salted provisions of Resolution N^o 12, dated of January 2, 2001 [5], Table 3 presents the counts of coliforms at 45°C, coagulase positive Staphylococci, *Salmonella*, molds, yeasts and halophilic bacteria during 90 days of storage. The enumeration of coliforms at 45ºC is used as an indication of the presence of potentially pathogenic bacteria of faecal origin, such as Escherichia coli. According to Brazil [5] the upper limit for the presence of this microorganism is 10^2 MPN/g. The samples of fish showed the absence of this microorganism during the storage period of 90 days. The presence of coagulase-positive staphylococci in foods is suggestive of the presence of *Staphylococcus aureus*, and its limit for dried fish and / or salted is $5x10^2$ CFU/g. During storage, the count of coagulase-positive staphylococci was $< 1x10^1$ in all evaluations, therefore within the limits established by law as the reference. With regard to *Salmonella*, it must be absent, since it is a pathogenic microorganism. In this study, there was no *Salmonella* in fishmeal throughout the storage period.

In brazilian law there is no limit set for halophilic bacteria in fish products, however, all samples were contaminated by these microorganisms, with a magnitude ranging from 10^2 to 10^3. These bacteria are responsible for the "reddish" deterioration known by this name because of the color of their colonies, defining the visual look of most of salted fishes. This contamination causes red pigmentation, and also produces an unpleasant odour and slime in the products [12, 13]. Vilhelmsson; Hafsteinsson; Kristjánsson [14,15], studying salt cod observed the appearance of vermilion in halophilic counts of 10^5 CFU/g. Lourenço, Sousa & Silva [16] also observed "reddish" deterioration scores higher than $3x10^6$ CFU/g in pirarucu sold in the city of Belém.

Despite the fact that contamination was present in the fishmeal type "piracuí" at all storage times, there were no characteristic colonies of these bacteria, taking into account their decay products as colonies characteristically red, slime and unpleasant odour. Presence of yeasts and molds was observed only at 15 days of storage with the count of 3.0 $x10^2$ CFU/g. For other storage times these microorganisms were not detected in fishmeal type "piracuí", because the presence of these organisms is not expected in the range of water activities that occurred during storage. This count alone may indicate contamination in some stage of evaluation, or even failure during the stage of product packaging. During the 90 days of storage, the fishmeal type "piracuí" proved to be microbiologically stable to pathogenic microorganisms, but was susceptible to the action of spoilage bacteria and halophilic bacteria that were present during the entire period of evaluation, although we have not noticed signs of deterioration in the product.

3.2 Adsorption isotherms

The equilibrium data of adsorption of moisture for fishmeal type "piracuí" at 25°C set by the BET model, are presented in Table 4 and Figure 1. It is noteworthy that during the obtention of adsorption data, through sensory inspection, were noticed changes in product characteristics such as loss of fluidity and intensifying of flavor. The fishmeal type "piracuí" showed isotherms of type III, according to the IUPAC classification [17]. The same behavior was observed by Molina-Filho et al. [18] obtaining sorption isotherms to tambaqui fish osmotically pre-dried. Typically, protein-rich products such as fishmeal type "piracuí" (74.65%) have type II isotherms, as shown by Assunção & Pena [7] studing dry pink shrimp. The addition of salt in both the fish meal type "piracuí" as the tambaqui meat tambaqui may have influenced the characteristic of the isotherm.

Table 4. Adsorption data for fishmeal type "piracuí" at 25 ° C

A_w	M (m H_2O/100g m.d.)	Aw	M (m H_2O/100g m.d.)
0.048	0.58	0.485	7.66
0.089	1.23	0.567	9.59
0.123	1.68	0.645	14.87
0.168	1.93	0.680	18.06
0.231	2.56	0.719	22.33
0.260	2.81	0.816	34.16
0.287	4.31	0.833	37.10
0.330	4.71	0.842	39.12
0.380	5.78	0.883	45.93
0.423	6.49	0.900	58.39

Averages of two determinations

It was observed that the adsorption isotherm of this product showed a linear response to intermediate levels of Aw, assuming an exponentially greater than 0.6 indicating Aw, that in an environment with humidity above 60%, this product requires greater care, because it adsorbs water easily and can be more susceptible to degradation reactions and proliferation of pathogenic microorganisms.

Based on adsorption data in Table 4, the product microbiological stability (Aw < 0.6) is present values of humidity below 10 g.100 g^{-1} d.b. However, when it made the relationship of the adsorption isotherm with the study of shelf-life, it was found that even in water activity below 0.6 occurred growth of halophilic bacteria, i.e. the data offered by isotherm does not provide microbiological stability of fishmeal type piracuí for this type of organism, since they can develop even for very low values of water activity.

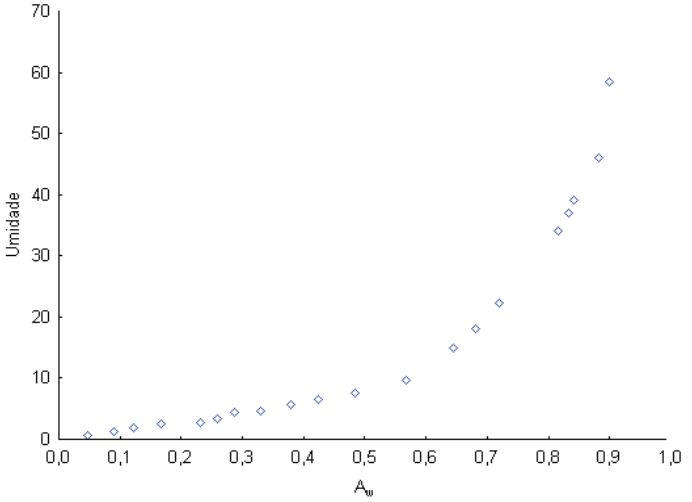

Fig 1. Adsorption isotherms for fishmeal type "piracuí" stored at 25°C

3.3 Prediction of adsorption isotherms

Table 5 show the coefficients of determination (R^2) and average relative deviations (P), used to evaluate the fitness of data for adsorption of fishmeal type piracuí the mathematical models. Analyzing the models as a function of R^2 appears that everyone had a great adjustment for predicting isotherm of fishmeal type piracuí by presenting determination coefficients near one ($R^2 \approx 1$).

The analysis of the relative mean deviation (P) showed that all the tri-parametric models showed good fit of experimental data, and the BET model presented the best fit. Among the bi-parametric models, it was observed that they showed a great variation between the values of P and the Oswin model which is closer to the value suggested by Lomauro, Bakshi and Labuza [19] who consider those good fits for which the P value is less than 10.

Table 5. Coefficients of determination (R^2) and average relative deviations obtained through the adjustment.

	Equation name	R^2	P
Tri-parametric Model	GAB (a)	0.9975	9.82
	BET (a)	0.9972	6.96
	Anderson (b)	0.9975	9.82
	Anderson & Hall (b)	0.9975	9.82
	Gascoyne & Pethig (b)	0.9975	9.82
Bi-parametric models	Halsey (a)	0.9935	40.38
	BET Learizada (b)	0.9946	12.73
	Owsin (a)	0.9967	10.43

(a) Park & Nogueira [10]; (b) Boquet et al [11]

4. Conclusion

The fishmeal type piracuí showed good physical stability and sensory, for the study of shelf-life, despite the presence of the halophilic bacteria during 90 days of storage. The results of the adsorption isotherm of the product showed that the water activity of less than 0.6 used in this study were not sufficient to inhibit the growth of halophilic bacteria. The tri-parametric model of BET presented the best fit to the experimental data.

Acknowledgements

The authors are very grateful to CNPq for financial support.

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Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS1006.